

chromatin structure may be "opened" by SWI/SNF complexes recruited by chromatin - FA protein complex (FANCA) - BRG1 interactions. On the other hand, during M phase, the SWI/SNF complex may be excluded from chromosomes, thus causing the chromatin to become condensed. The physiological action of FANCA on the SWI/SNF complex remains to be clarified, but our work suggests that FANCA may recruit the SWI/SNF complex to target genes, thereby enabling coupled nuclear functions such as transcription and DNA repair.

#### Abstract# 3254

**Fanconi Anemia Type C and p53 Cooperate in Cell Cycle Control, Tumorigenesis, and Development.** Brian Freie\*,<sup>1</sup> Samantha Ciccone\*,<sup>1</sup> Xiaxin Li\*,<sup>1</sup> Artur Plett\*,<sup>1</sup> Christie Orschell-Traycoff,<sup>1</sup> Edward Srour,<sup>1</sup> Helmut Hanneberg\*,<sup>2</sup> D. Wade Clapp.<sup>1</sup> <sup>1</sup>Depts. of Microbiology/Immunol, Peds, Med., Lab. Animal Res. Center, Biochemistry, Herman B Wells Res. Ctr., Indiana University School of Med., Indianapolis, IN; <sup>2</sup>Dept. of Pediatrics, Univ. of Dusseldorf, Dusseldorf, Germany.

Fanconi Anemia (FA) is a heterogeneous genetic disorder composed of seven complementation groups (FANCA-FANCG) that are phenotypically characterized by progressive bone marrow failure, a risk of malignancies, and congenital anomalies. The cellular functions of this gene family are incompletely understood. While the propensity for chromosomal breaks in FA cells implicates these proteins in DNA repair, it does not exclude a role in other cellular processes such as cell cycle control. Cell cycle controls ensure proper cell cycle progression and are critical for maintenance of genomic integrity. Though most studies of FA cells have emphasized the use of mitomycin c to study FA cellular abnormalities, there is increasing evidence that FA proteins modulate cell responses to ionizing radiation (IR) given that FANCG is the human homologue of XRCC9 and FANCD2 undergoes posttranslational modification in IR treated cells. IR induces both a G1 and G2 arrest in wildtype cells. To test the hypothesis that the murine homologue of FANCC (Fance) is deficient in IR induced checkpoint arrest, the cell cycle kinetics of a defined population of wildtype and Fance mutant murine embryo fibroblast cells (MEFs) were monitored through G2 and M phases of the cell cycle following 0-10 Gy of IR. Consistent with previous studies, we found that wildtype cells had a sustained arrest for 24 hrs in G2 following IR treatment. However, up to 40% of Fance<sup>-/-</sup> cells escaped G2 16-20 hrs following IR, indicating that these cells were unable to maintain the G2 checkpoint. A similar G2 escape was observed when primary, cutaneous fibroblasts from patients with FANCC were examined. Maintenance of the G2 checkpoint requires the p53 dependent down-regulation of cdc2 levels. Therefore, we assayed cdc2 protein levels and p53 function in Fance<sup>-/-</sup> MEFs. As anticipated, cdc2 levels decreased in wildtype MEFs following IR, but remained elevated in Fance<sup>-/-</sup> cells. Elevated cdc2 levels in Fance mutant cells were observed despite adequate p53 protein levels and p53 DNA binding activity, suggesting that Fance either functions independent of p53 or impacts a common biochemical pathway downstream of p53 to regulate cdc2 levels. To differentiate these possibilities, Fance and p53 mutant mice were intercrossed, and MEFs from F2 progeny were analyzed. Following IR, there was an additive increase in G2 escape in fibroblasts that were mutant at both Fance and p53, indicating that Fance and p53 cooperate in G2 checkpoint control. To examine whether Fance and p53 cooperate in development and tumorigenesis, large cohorts of F2 progeny were monitored (17-25 per genotype). Mice mutant at Fance alone do not develop tumors. However, compared to mice mutant at p53 only (which develop tumors at a high rate), mice that were mutant at Fance and p53 had a reduced latency in tumorigenesis. Furthermore mice that were nullizygous at both Fance and p53 had an increased incidence of *in utero* lethality. Collectively, these data indicate that Fance has a function in checkpoint control, and that this control occurs cooperatively with p53. The data also indicate that Fance and p53 cooperate in tumorigenesis and development.

#### Abstract# 3255

**Recruitment of Alpha Spectrin II, the FANCA Protein and the Repair Protein XPF to Nuclear Foci in Normal but Not FA-A Cells in Response to a DNA Interstrand Cross-Linking Agent.** Muriel W. Lambert, Laura W. McMahon\*, Deepa Sridharan\*, W. Clark Lambert\*, Monique Brown\*. Pathology, UMDNJ - New Jersey Medical School, Newark, NJ.

Fanconi anemia (FA) is a genetic disorder characterized by aplastic anemia, cancer susceptibility, hypersensitivity to DNA interstrand cross-linking agents and a defect in ability to repair this type of damage. We have identified, in the nucleus of normal human cells,  $\alpha$  spectrin II ( $\alpha$ SpII\*) as a component of a chromatin-associated protein complex involved in repair of DNA interstrand cross-links, and have shown that it forms a complex with the FANCA, FANCC and FANCG proteins, binds directly to DNA containing interstrand cross-links indicating a role in the repair process, and is deficient in FA-A, FA-B, FA-C, FA-D, and FA-G cells. The present study examined the localization of ( $\alpha$ SpII\*), FANCA and XPF, a protein involved in repair of DNA interstrand cross-links, in cells after treatment with a DNA interstrand cross-linking agent, 8-methoxypsoralen plus UVA light (8-MOP). Using immunofluorescence microscopy and a double labeling technique, cells were either treated with 8-MOP or untreated, probed with primary antibodies (Abs), followed by staining with the appropriate fluorescent-conjugated secondary Ab. The results showed that in undamaged normal cells, ( $\alpha$ SpII\*), FANCA and XPF were present in a diffuse pattern in the nucleus. After treatment of these cells with 8-MOP, ( $\alpha$ SpII\*) relocated to discrete damage-induced foci distributed throughout the nucleus and FANCA and XPF co-localized to these same foci. In contrast, in FA-A cells, either damaged or undamaged, only very faint staining of ( $\alpha$ SpII\*) was observed in the nucleus and no FANCA was visible. XPF, which is not deficient in FA-A cells, was present in the nucleus of undamaged FA-A cells but did not relocate to discrete nuclear foci following damage. In FA-A cells, transduced with a retroviral vector expressing the [FANCA] cDNA, ( $\alpha$ SpII\*), FANCA and XPF again co-localized in discrete foci in the nucleus after damage. These results indicate that ( $\alpha$ SpII\*) plays a pivotal role in the recruitment of FANCA and XPF to nuclear foci after cells are damaged and that in FA-A cells, where there is a deficiency in ( $\alpha$ SpII\*), this

recruitment does not take place. ( $\alpha$ SpII\*) may act as a scaffold to aid in targeting repair and FA proteins to sites of damage, enhancing their interactions. In FA-A cells, where this scaffolding is deficient, these interactions are defective. This correlates with the reduced repair of interstrand cross-links that occurs in FA-A cells.

#### Abstract# 3256

**Clinical Phenotype of Fanconi Anemia Is Influenced by Polymorphism in Glutathione-S-Transferase Theta.** Stella M. Davies,<sup>1</sup> Gretchen A. Radloff\*,<sup>1</sup> Todd Defor\*,<sup>1</sup> Sat Dev Batish\*,<sup>2</sup> Philip F. Giampetro\*,<sup>2</sup> Arleen D. Auerbach.<sup>2</sup> <sup>1</sup>Pediatrics, University of Minnesota, Minneapolis, MN, USA; <sup>2</sup>Laboratory of Human Genetics and Hematology, The Rockefeller University, New York, NY, USA.

Fanconi anemia (FA) is a monogenic autosomal recessive disorder associated with chromosomal instability, marrow failure, and a range of congenital malformations. The clinical manifestations of FA vary significantly between affected siblings, indicating that genetic background may significantly contribute to FA phenotype. Abnormal oxygen metabolism is a hallmark of FA cells, due to either excess generation or decreased removal of reactive oxygen species. Glutathione-s-transferases (GSTs) are a family of Phase II enzymes that catalyze the conjugation of glutathione to xenobiotics. GSTs also possess glutathione peroxidase activity and play an important role in the detoxification of lipid and nucleic-acid hydroperoxides produced during oxidative stress. We have previously shown that absence of the GST theta gene (GSTT1) increases *in vitro* sensitivity to DEB in FA patients (Davies et al, Blood, 94:408a,1999). Absence of GSTT1 is a frequent population polymorphism, occurring in approximately 20% of white and 30% of black people. We have investigated polymorphisms in the GST genes GSTT1 and GSTP1 as potential modifiers of the FA phenotype. We hypothesized that absence of the GSTT1 gene would be associated with a more severe phenotype, as these individuals show greater DEB sensitivity. We compared frequencies of major congenital malformations and time to onset of hematologic disease in 158 individuals with FA registered with the International Fanconi Anemia Registry (IFAR). The median number of malformations is increased in GSTT1 negative (n=29) compared with GSTT1 positive (n=129) cases (median 2 vs 1 abnormality, p=0.08). Comparison of frequencies is shown below.

Genotype	0 Malformations	1 Malformation	>1 Malformation
GSTT1 positive	47 (36%)	27 (21%)	55 (43%)
GSTT1 Negative	6 (21%)	4 (14%)	19 (66%)

Median time to onset of hematologic disease is 4.6 years (range 1.8-21 years) in GSTT1 negative cases and 6.8 years (range 0-26 years) in GSTT1 positive cases (p=0.16). Recent data have shown that the Fanconi anemia group C protein (FANCC) interacts with GST P1-1 (GSTP1) and augments its activity during apoptosis (Cumming et al, Nature Medicine, 7, 814-820, 2001). The GSTP1 gene contains 2 single nucleotide polymorphisms in exons 5 and 6, producing 3 possible alleles, GSTP1\*A, GSTP1\*B and GSTP1\*C, which have been shown to have markedly different kinetic properties and substrate specificities. Analysis of DEB sensitivity, number of congenital malformations and time to hematopoietic disease all showed no associations with GSTP1 genotype. Analysis of only complementation group C patients also showed no association with GSTP1 genotype. We conclude that GSTT1 but not GSTP1 genotype influences clinical phenotype of FA. This is the first demonstration of modification of the FA phenotype by genetic background. This is also the first demonstration of modification of susceptibility to congenital malformations by a frequent population polymorphism in a detoxification enzyme.

#### Abstract# 3257

**Cancer in Fanconi's Anemia: A North American Pilot Survey.** Blanche P. Alter,<sup>1</sup> Philip S. Rosenberg\*,<sup>2</sup> <sup>1</sup>Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD; <sup>2</sup>Biostatistics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD.

Patients with Fanconi's Anemia (FA) have a high risk of leukemia and solid tumors. The objective of this study was to identify the types of cancers for which FA patients are at risk, quantify those risks, and determine associated risk factors. Among >1200 FA cases in the medical literature, 103 had leukemia, 59 had non-hepatic solid tumors, and 34 had liver tumors; 17% had at least one cancer. Since literature reports are subject to publication bias, a cross-sectional prevalence survey was conducted in US and Canadian families who belong to the Fanconi Anemia Research Fund and Fanconi Canada, respectively. 145/318 questionnaires were returned, a 46% response rate. Sixteen percent of patients (26 cancers in 23 individuals) had a history of cancer (including leukemia and excluding myelodysplastic syndrome). Cancer diagnoses were confirmed using medical records, pathology reports, or death certificates. There were 9 cases of leukemia, 6 head and neck cancers, 2 each esophageal and liver, 3 vulvar, and 1 each of cervical cancer, osteosarcoma, soft tissue sarcoma, and brain cancer. The cumulative probability of developing a specific complication was determined with the method of Kaplan and Meier. The risk of leukemia reached a plateau of 20% at 25 years of age, and the probability of non-hepatic solid tumors was 75% by age 45 without a plateau. The Connecticut Cancer Registry was used to determine the relative risk of cancer in FA compared to the general population. The cancer-specific observed/expected ratios after adjustment for age and sex were significantly and substantially elevated: 774 for acute myeloid leukemia, 675 for head and neck cancer, 2115 for esophageal cancer, 377 for liver tumors, and 4031 for vulvar cancer. Patients who developed solid tumors had a later diagnosis of FA than those who did not develop tumors (median age 8 vs 5 years, p<0.01). The data are consistent with the interpretation that patients with the more severe FA phenotype develop early aplastic anemia, and may not survive long enough to experience the later complications of leukemia and solid tumors. FA patients have a very high risk of cancer, and can serve as a model for investigation of mechanisms of carcinogenesis in humans. These families also comprise a group at high-risk of cancer who may benefit from focused cancer screening and prevention programs.